

January 25, 1947.

Test types of 407 in BMIB, (-L) and in very light media of the non-purified culture.

		Streak out colonies of 407 in EMB-lactose Test on:	
		BMIB	BMTL
1.	407 -	+	+
2.	2 +S	+	+
3.	3 +S	+	+
4.	4 +S +R use a colony in line of vein streak (may be mutant from S to R).	+	+
5.	6 +S do.	+	+
6.	7. +S	+	+
7.	7. +S	+	+
8.	10. +S	+	+
9.	11 +R	+	± P
10.	12 +R.	+	+
11.	13 +R	-	-
12.	13 -R	+	+
13.	13 -R	+	+
14.	14 +R	+	+
15.	15 +R	+	+
16.	16 -R	-	+
17.	16 +R	+	±
18.	18 +R	+	+
19.	20 +R	+	+

Transfer 10, 11, 12 to slants + test further.
16, 17

3-way crosses.

January 27, 1947

1. $\text{BM} + R \swarrow \begin{matrix} \text{TLB}, -R \\ \text{TLB}, +S \end{matrix}$

$\text{Y}64$	$\rightarrow -R, +R$
$\text{Y}10$	$\rightarrow +R, +S.$

$\text{Y}40$

$-S$	$-R$	$+S$	$+R$
0	16	7	28

 $\boxed{51}$

Therefore one can assume that
an error was made in the
previous experiment.

See 368, ~~42~~ 398.

$58-161 \swarrow \begin{matrix} \text{Y}10/1 \\ \text{Y}53 \end{matrix} \rightarrow \begin{matrix} 38-S \\ 16+R \\ 0+S. (\text{rare!}) \\ 0-R \end{matrix}$

January 27, 1947

BM+S TLB, -S Y53 → -S, +S
58-161. TLB, +R Y46 → +S, +R.

-S -R +S +R
3 0 0 0.

Tests for > 2 strand crossovers.

Tests for phenotypic lag.

January 27, 1947

1. Plate Y40 + Y53 in B, agar.

(Use colonies of 407) stand in cold room.

a. Streak out on EMB agar. & b. plate remainder of colonies in T(O) agar.

Use well separated colonies
here.variability in $\times 10^3$ $\times 10^3$

0/3

0-turbid

$Y40 \times$	1	-	0/3	colonies
$Y53$	2	-	0/3	
and A	3	+	0/3	
	4	+	0/3	
	5	+	0/3	turbid
	6	-	0/3	
$Y64 \times$	11	-	0/3	turbid
$SP \cdot 161$	12	-	0/3	
and B	13	-	0/3	colonies
	14	-	0/3	
	15	-	0/3	ca 20 colonies!
	16	-	0/3	0

b. Streak out 18 other colonies on EMB, looking for variation.

A	1	+	0/10 ³	
	2	-	0/3	
	3	-	0/3	
	4	-	0/3	
	5	-	0/3	
	6	#	ca 1/2 each.	
	7	-	0/3	
	8	-	0/3	
	9	-	0/3	
			0/3	
			0/3	
B			0/3	
			0/3	
		+	0/3	
		-	0/3	
		0/3		
		0/3		
		0/3		
		0/3		
		0/3		
		+	0/3	
			<u>0/3</u>	

Total: $6 + 13 = 80\%$

only 1 heterogeneous.

January 27, 1947

1. Y57 x Y68 (check on previous datum) No colonies

2. Y64 x Y68 in B,
Y53/M x 58-161/M No colonies!

3. Y53 x Y68 (test for recombinaibility). No colonies

- 4. Y67 x Y40. in B, very numerous, very elongate colonies.
Y53/M x 58-161/M

- 5. Y68 x Y53. in B, Fair, v. elong. colonies.
Y53 x 58-161/M

6. Y67 x Y68 in O. No colonies

7. Y67 x Y69. in O. scattered few (< 10⁻⁸) colonies. all dry.
alleles.

1	Y53 x 68	n.g.
2	Y57 x Y68	n.g.
3	Y64 x Y68	n.g.
6	Y67 x Y68	n.g.
4	Y69 x Y53 Y40/M Y53/S	OK.
5	Y40 x Y67 Y68/S x Y53/M	OK.
7	Y67 x Y69	OK, but poor.

Y67 = Y53 M

Y68 = 58-161 M

Y69 = Y40 M

January 30, 1947.

Pedigree:

Received from Dr. Max Zelle, N.I.H., Bethesda, Md.

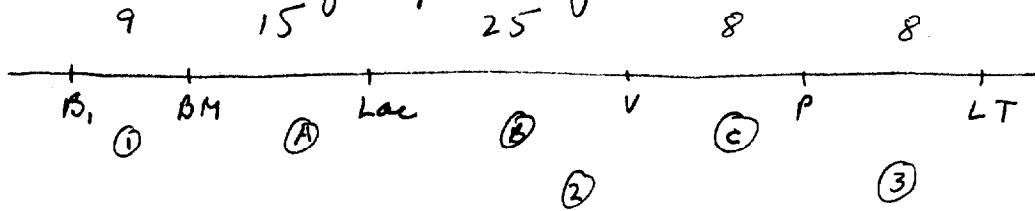
Var V₁.

B - S

C - S
- S
- S
- S
- S

D - S
- S
- S
- S
- S
- S
- S

On basis of map theory:



1. Prototrophs are ①③ types or ①③②.
By the cross: BP × Y64:

-	+	-	R	+	-
+	-	+	S	-	+
①	②	③	④	⑤	⑥

the most frequent prototroph class, by far, should be -R.

The other types all require double crossovers. The relative frequencies of types should be of the order of:

-R	.87
+R	.08
+S	.03
-S	.04

The position of P is inferred from rather complex data. It can be confirmed by showing that LT does not affect the segregation, nor app. increase the yield. If Chr. B₁⁻ may run + it would likewise be difficult to use only B₁ as the marker.

a) B₁⁻ > B₁⁺, S influence on segregation.

B₁⁻ > B₁⁺ = following distribution (ca.)

-R	.30
+R	.50
+S	.15
-S	.05

BLT, B₁P, LT, should be readily recoverable.

BP x TG

416

PLATE

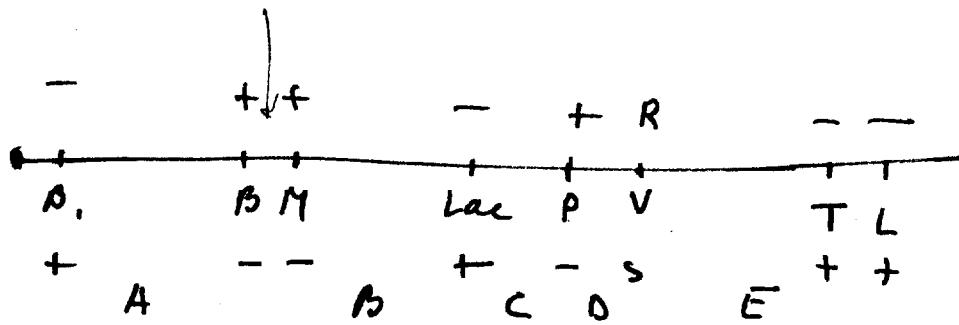
To demonstrate genetic as well as biochemical
distinctness of prolineless (glut -) and prolineless (glut +)

58-5255 x 679-662

Pattern small moles. / (10^8)

8 colonies found.

✓



$\frac{1}{2}$ B.
+ - -

REFE

58-5255 x 464.

1. Plate 5255 in O, Biotin alone for R test.
 O: no colonies.
 B: " "
2. Plate into O, B₊; B₋.
 B₊ - too turbid } use more dilute
 B₊, P. inocula
 BLT - very turbid, but several colonies
 recoverable
- B₊ > O. (5-10 x)

B ca 2-3 x O.

Photographs:	-R	-S	+R	+S	Total	Total -
from B ₊ plates:	25	27	0	4	56	
	62	41	2	1	106	.93
	1.8	3.1	.0	.1		.97
from B ₋ plates	74	69	10	12	167	.85

$$\chi^2_3 B/B_+ = 11.1$$

of which 8.3 is +R, +S classes. ∴ B is certainly modifying the distribution

on basis of map B₊ B₋ lac V P TL, the phototroph distribution should have been predominantly -R, while B₋ shows 1/2 +R, which is not suggested by these data. Also would be better data on the frequency of B₋/B₊. This distribution suggests the map order:

B₊ B₋ lac P V TL.

Lac^R × Lac+ ~~#~~
Y76 × Y40.

418

7 FEB

B.⁻ 38/35 Lac+

B.⁺ 10/10 Lac+. 45 tested all Lac+

Streptomyces Resistance

419

7552

Recd. 200,000 Units of streptomycin HCl, Meckle, Lot 277
 Potency 250 U/mg officially from H. Robinson.

A. Dissolve 100,000 units in 10 ml H₂O for stock solution: 10⁸/ml
 Dilute serially for stocks of 10³ & 10²/ml.

Use 10⁴, 10³, 10² u./plate ± controls on washed Y53
 (standing 24 hours in H₂O per.).

10 ⁴	-
10 ³	-
10 ²	ca. 100, very small "resistant" colonies at 18 hours. Incubate further.
0	+++T
0	+++T.

Recotypes - see 907

409

January 25, 1947.

Test types of 907 (in BMTB, (-L) and in very light media of the year-
 previous culture).

Streak out colonies of		907 in EMB-lactose-T test:		P
		BMB	BML	
1.	407-	+	+	{ P
2.	2+S	+	+	
3.	3+S			
4.	4+S+R in line of virus streak (may be resistant from S to R).	+	+	
5.	6+S do.	+	+	
6.	7. +S	+	+	
7.	10. +S	+	+	
8.	11. +R	+	±	P

Deoxyribonuclease and recombination.

450.
420

7/17/67

Receive 10 mg DNase (gift of Avery, MacLeod) in 10 ml 2x coli minimal. Sterile filter - filtered well. Store in cold. Preserve no denaturation.

Plan: Add .1 ml of DNase (1 mg/ml) to 1 ml of cell suspension, separately. ~~to~~ Mix cell suspensions + plate $\frac{1}{2}$ ml. Also, hold cells in DNase, in minimal medium

A.	.1 ml	Total 200 v/4 plates	17, 8, 19, 15	Av. 15 ✓
B.	.5 ml ea.	2 mg/4 plates	6, 11, 12, 33	15.5 ✓
C	control		9, 7, 23, 13.	13. ✓

In this expt., DNase has had no appreciable influence on recombination.

3 mg 80 S or 100

Dedicate T L ✓

421

2/10/47

BT/I x B,L.

i.e.

		42	16	
B ₁	B ₂	V	T	L
+	-	S	+	-

Prot:

S > R.

↑

↑

↑

T⁻

R > S

↑

T⁻ >> T⁺

L⁻

S > R

↑

T

L⁻ < T⁻ > L⁺

or.

	B ₁	B ₂	V	L	T
	+	-	S	-	+
	-	+	R	+	-

Prot: R > S.

↑

↑

T⁻

R > S.

↑

L⁻

↑

↑

Plate mixture into O, T, L.

O: 1/4

T 1

L 1.

e.g. at recombination.

Three-way cross.

422

February 10, 1947

Y64 x Y10 x Y40. -

Yield very poor. Do not use ~~with~~ for testing.

cf. other experiments this date!!!! - minimal medium??

to February 10, 1947

Repeat part B and controls of 420. 480 x 853.

B: $\frac{1}{2}$ ml cells + $\frac{1}{2}$ ml DRNase separately + mix cells. Plate into minimal + B, agar. ($\frac{1}{2}$ ml of mixture).

B: 0 20
B. ca 150.

C: 0 2
B. 8

controls did not do well here!
(agar base cloudy!)

Test various polynomials - vs
against Y41, etc. for gene homology.

424

1. Feb. 10 '47.	58-3214 x	Y41.	No. prot. / 10 ³
2.	Febr. 13		0
	6177		0
	3232		0
	6049		0
	6317		0
	5450		0
	5255		0, 0

679-448 x 5255

0

Test Koepke's mutants for recombination.

425

February 10, 1947.

24h. 486

1. Thr — 5
x
bird

2. meth 2000 $10^{3.5}$
x
arg

3. lys ~~thr~~ 0
x
leuc

4. pro ~~thr~~ 0.
x
cit-uracil ~~thr~~

5. ~~met~~ meth
x
Y64. —, —, — later 3/4 plates!

6.	thr	—	0	558-228
7.	bird	—	5	1250-228
8.	meth	—	2	532-171
9.	arg	2000	$10^{3.5}$	572-228
10.	lys	—	40	8152-171
*	leuc	—	1 (probably contam!)	45
12.	pro	—	100	209-301
13.	cit-ur. thr	—	0 (stab)	823-304

#

no evidence of recombination.
threon + leuc seem to be most stable types in
this series.

liverporosis vi Y53.

Febr. 10, 1947.

Treat cell suspension of Y53, \bar{v} .01% HN2 (bis- β -chlorethyl methylamine-HCl) in phosphate-citrate buffer, pH 6.0 for 1/2 hour at room temperature. Terminate treatment by diluting with broth + centrifuge + wash into fresh YB broth. Incubate overnight.

- A. Streak out EMB agar after 4-hour incubation.
 Y53 has proliferated considerably; Y40 has not!
 Take isolated colonies to YB liqu. | do. below.
 Y53: 21-40 Y40-1-20.

- B. Streak out cultures after 20 hour incubation.

Also, take slants from entire population.

- P 13. Cross 10 cultures from A, each, \bar{v} Y40 + Y76 resp. in

O + B₁ agar.

	O	B ₁	O	B ₁	O	B ₁	O	B ₁
1	10	100	11		21	20	100	31
2	14	100	12		22	10	100	32
3	26	800	13		23	60	300	33
4	15	100	14		24	20	500	34
5	20	100	15		25	8+	100	35
6	0	60	16		26	14	50	36
7	1	100	17		27	20	100	37
8	2	100	18		28	40	500	38
9	7	30T.	19		29	50	500	39
10.	3	50	20		30	10	500	40

all OK.

[Repeat 6, 7, 8 +] ^{6?} 0
 [compare \bar{v} app. controls.]

Reversion

427

Febr. 13, 1947.

$Y76 \times 58-161$. in $T(0) + T(B_1)$

$B_1 - 44$ lac⁺

$B_1 + \frac{9}{53}$ lac⁺

add to 418: 45 tests.

= 98 tests.

This tests for only 49 recombinations,
since $\frac{1}{2}$ would be $E^+ lac^+$.

Resistance.

428

Feb. 13, 1947

1/2 ml eq. 426. per plate: VSA.

Streptomyces —

10 u.	turbid plate.
50 u.	As below.
100 u.	ca 10^2 small resistant colonies. (did not mix adequately in agar).
1000 u.	med > 5 u/ml.

Brilliant Green (1:1000)

1 ml	-
.5 ml	-
2 ml	no resistants found!
.1 ml	turbid.

 $HgCl_2$

10 mg	-
1 mg	-
.1 mg	not well diffused; evidence of resistance in some regions.

Tyrocidin (in alcohol)

500 v	turbid (ca 10^7 colonies)	= 50 v/cc no inhibition.
200 v	do.	
100 v	do.	

∴ Tyrocidin 1 mg.

B.G. OK at ca + 1: 100,000

Mg OK at ca 5 v/ml.

Streptomyces OK at 5-10 u/ml.

Fermentation tests.

428

February 17, 1947.

Repeat -

[Used mustard treated cultures].

strike out Y40, Y53 on sugar-EMB media.

Y40 Y53

Lactose * ++ -

* some - colonies? -
pink + red.

Maltose ++ +++

Mannitol variable variable, predominantly -. later - pink +

Glycerol - (+). variable late +! Note diff. Y53 & Y40.

Alcohol ±

Sucrose - ± - ± (faint blue coloration not a + reaction).

Citrate pH too low

Note 3/18: Xylose: K12 is ++

Maltose is definitely +.

Sucrose seems distinctly - - select for + mutants??

Mannitol + glycerol may be too variable to be useful.

Sucrose - = E. coli communis.

Inversions in Y40.

429

February 18, 1947.

Cross 426-6, 7, 8 = Y53; Y76 = + S B.

A

B

X Y76

X Y53

1 426-6. 40, 50 ^O ca 200, ✓ 50, ✓ ^O 200T, ✓

2 426-7 ca 50. 400, 50 500

3 426-8 150, HT; 150, HT;

4 ~~SP-161~~ 100T HT; 200, HT

~~SP-161~~
(no Y40m).

All inversions

Inhibitors

430

February 18, 1947.

per "20" ml plate VSA.

Y64 Hg 100v "I resistant colony. not too well diffused
 Y46 do. clear. - "secondary colonies!!"

Y64 Streptomycin 50u turbid
 100u. clear in parts. Not wildest.

58-161. 50u. turbid
 100u. as above.

Y64. Brilliant Green .5ml 1:1000 clear! (1:40,000). ~~2~~ pick up resistant
 " Malachite Green 1:500 1ml clear! (1:10,000). ~~ca 2 large~~; 40mm.
 .5ml " 1:20000
 .1ml (1:100,000). poorly diffused.
 elsewhere def. is visible

Y46. Sodium Aride 10v turbid
 100v turbid
 1mg. irregular clearing.

Y11 A6 from glucose

Y64 "Spengon" (1/20) 1% no inhib.

Y64 tri-codolamine (1/20) 1% "

58-161? Hg Cl₂, 1mg. autoclaved with 100ml N.A. — not inhib.

" Bi. Cl₂. 2mg/100ml N.A. — ~~inhibit~~ only faintly inhib.
 = 1:50,000. (and more!)

" Streptomycin 5u/ml. OK.

Y64 NaNO₃ 2ml/20 .1% inhib. — later grew uniformly.

Y64, 58-161. Mal. Gr. 2mg/100 NA — not enough!

Sex in L15 mutants?

February 17, 1947.

Grow separately; plate together.

Y5	2
"then.	0
Y5×then	0.
Y5×Y44	0
Y5×58-161.	0

no evidence for sexuality under these conditions. Try growing together!

440 x 453.

432

Re-suspension

2/21/47

Mix 440+453 in water, add to agar, mixing. Add 5 ml aliquots
 \approx ca. 1 mm. to various suppl. plates.
 (see 433 for note on medium).

5 ml.	0	12	B	"	10
		10			4
		10			15
		3			
		11			
		10.			10.

B.
 > 25 T.
 "
 "
 "
 "

This expt. illustrates influence of conditions on detection of recombinants.

10 ml - 5 sublays.

0 —
 0 —
 0 —

5 sublays. 0 5
 0 4

B. T.

B. 12.

Tests for division in B_1 -B regions.

433

2/21/47.

Cross mustard treated (426) isolates of Y40, Y53 ±

Y53 × Y40 resp. in O, B_1 medium resp.

Plate in
medium lacking NH_4NO_3
(inadvertent omission).

Y40T.

	O	B_1	
11	++	T	++
12	++	T	++
13	++	T	++
14	++	T	++
15	T	T	++
16	++	T	++
17			
18	++	T	++
19	++	T	++
20	++	T	++

no evidence of division in any
of these isolates —

$$17+20 = 37 \text{ tests.}$$

Y53T.

31	++	T	++
32	++	T	.
33			
34	++	T	++
35			
36	++	T	
37	++	T	
38	++	T	++
39	++	T	++
40	++	T	

number of prototrophs on this minimal seems quite unusually
high.

In tube test tubes, Y40 × Y53 all spp. inhibited by anaerobic conditions